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1. Your reference

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2. Patent application number
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0208041.4

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Lonza Biologics plc.
228 Bath Road, Slough SL1 4DY

Patents ADP number *(if you know it)*

Berkshire/Great Britain 7827959001

If the applicant is a corporate body, give the country/state of its incorporation

United Kingdom

4. Title of the invention

Method of Culturing Animal Cells

5. Name of your agent *(if you have one)*

O'Reilly Deana

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Lonza Biologics plc.
Legal Office
228, Bath Road

Slough, Berkshire SL1 4DY / Great Britain

Patents ADP number *(if you know it)*

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Signature Dr Ronald Waibel Date April 8, 2002

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L.B.P. 28, Europe
Patent Application:

Original Application
Of:

Lonza Biologics plc., Slough

Cell culture medium

Description

The present invention relates generally to the field of animal cell culture. It devises a method of culturing animal cell for production of therapeutic or other useful proteins and a respective cell culture medium.

Sodium butyrate or other salts of butyric acid are well-known to enhance yield of protein produced in animal cell culture as is described e.g. in EP-239 292. The effect can be observed for naturally secreted proteins, e.g. antibodies from hybridomas, or recombinant cell lines. Butyrate is added to cell culture medium in a concentration of preferably up to 5 mM. The effect of butyric acid is rather specific as is confirmed by a wealth of scientific literature on addition of butyric acid to culture medium; propionate or pentanoate are considerably less effective at concentrations at about 1mM. Acetate testing has not been reported.

However, there are limitations and backdraws to the use of butyrate as a cell culture supplement. Addition of butyrate in the 1-10 mM range needs to be carefully balanced in order to avoid overdosing and the ensuing toxic and cytostatic effects. The negative effect on growth rate can be drastic even upon minor increases in concentration. For each cell line and recombinant clone, the optimal amount of butyrate has to be carefully chosen and controlled during large scale bioreactor cultivation. In as much, butyrate has proven to be a somewhat Janus-faced means for increasing producitivity in cell culture.

WO 00/39282 describes a process for production of a protein by cell culture, said process employing an alcanoic acid as a medium supplement that is said to be effective at a concentration of less than 0.1 mM. Data for proving such effect are only given for butyrate in relation to NS0 cells. However, the positive effect on yield in the 0 to 0.1 mM shows a positive correlation with the amount of butyrate added, as could have been expected. The yields are therefore less optimal than working with higher doses of butyrate.

US 5378 612 describes a synergistic, protein yield enhancing effect of adding lithium acetate (10mM) , lithium chloride (10mM) or lipopolysaccharide (LPS, 1µg/ml) to a culture medium for CHO cell culture already comprising sodium butyrate at 1 mM. For the two lithium salts, the yield effect was in the order of 1.3 times, whereas LPS showed an enhancing effect of about 4 times in comparison to the butyrate control. Interestingly,

sodium acetate at 10mM proved to be of literally no benefit over the control in this test system.

Kim et al. (Biotechnology and Bioengineering 71, 2001, 184–193, Overexpression of bcl-2 inhibits sodium butyrate-induced apoptosis in CHO cells resulting in enhanced humanized antibody production) countered the cytotoxic effect of sodium butyrate at 5mM by means of bcl-2 recombinant cell lines which measure resulted in enhanced protein production. However, this method requires extensive cell line engineering for creating recombinants producing both bcl-2 and a product protein. A more simple method would be desireable to counter the negative effects of butyrate medium supplements.

It is an object of the present invention to avoid the disadvantages of the prior art and to devise another method for enhancing the yield of product protein in animal cell culture.

This object is achieved according to the present invention by a method for culturing animal cells wherein a cell culture medium is supplemented with acetate. Another object of the present invention are the corresponding cell culture media comprising acetate.
Possible embodiments of the invention are shown in the figures and tables:

Fig. 1 shows the effect of potassium and sodium acetate on productivity in fed-batch shake flask culture.
Fig. 2 shows growth profiles for fed-batch bioreactor fermentations containing no or 10 mM sodium actetate
Fig. 3 shows the effect of 10 mM sodium acetate on productivity in the fermentation run according to Fig. 2.
Fig. 4 compares the effect of inclusion of sodium acetate in inoculum cultures (in inoc) and the time of further acetate addition (at inocculation or during mid-exponential phase, i.e. at feed). All values are expressed as percent difference compared to control.

According to the present invention, a method of producing a product protein is devised wherein the product protein is expressed from a mammalian cell in cell culture and is produced at least during a certain span of time during cell culture. That is, the product protein might either be constitutively expressed by the cell or expression might be induced at some point of time by providing a certain stimulus to the cell. Preferably, it is

constitutively expressed. The method according to the present invention further comprises the steps of

a) preparing a cell culture medium for mammalian cells,

b) and further adding acetic acid or an acetate salt or a biologically activated acetyl ester to a final concentration of from 1 to 20 mM, preferably of from 3 to 15 mM, more preferably of from 5 to 12 mM, most preferably of from 6 to 9.5 mM, said addition being carried out either directly to the medium prior to starting cell culture or feeding it to the medium during cell culture,

c) further culturing the mammalian cell in said medium with concomittant expression of product protein,

d) and finally harvesting said protein from the cell culture.

A product gene according to the present invention is the product protein that is sought to be expressed and harvested in high amount. It may be any protein of interest, e.g. therapeutic proteins such as interleukins or enzymes or multimeric proteins or submits of multimeric proteins such as antibodies or fragments thereof. The recombinant product gene may include a signal sequence coding sequence portion allowing secretion of the once expressed polypeptide from the host producer cell. The product protein may be a recombinant protein expressed from a transgenic promoter or it is a naturally active gene locus, such as e.g. an immunoglobuline gene locus in hybridoma cells created by conventional cell fusion techniques. Harvesting and downstream processing techniques for purification of the protein from the culture broth are well-known in the art and a routine task. Initially, techniques such as centrifugation, ultrafiltration and/or ion exchange chromatography are often applied since they allow for high-volume throughput.

An acetate salt according to the present invention can be any salt such as alkali or alkaline earth alkali or any other metal acetate salt may be employed. Since media are usually buffered, addition of acetic acid or acetic anhydride is also conceivable. Likewise, easily dissociable complex salts of acetate or esters of acetic acid that are liberating acetate in situ in the culture medium during culture, either due to a high rate of hydrolysis or due to activity of cellular exoenzymes, can be employed. Such can be termed, in particular if applying to exenzyme activity, 'biologically activatable' esters. Alkali and alkaline earth metal salts of acetate are preferred embodiments of the present invention. More preferably,

the salt is an alkali metal salt with the proviso that the alkali metal is not lithium, most preferably, it is sodium acetate. Sodium acetate is particularly preferred in combination with a concentration range of 6 to 9.5 mM, a cell growth medium as being opposed to a maintenance medium and upon treatment of the cells with sodium acetate at the onset or prior to the onset of cell culture or both, as set forth below.

Preferably, the cell culture medium of the present invention is devoid of butyrate. Butyrate easily decreases growth rate and induces apoptosis; its concentration needs to be carefully balanced. However, according to the present invention, it can be easily substituted by acetate showing almost no or a very moderate effect on growth rate. Acetate is not known to induce apoptosis.

According to the present invention, acetate or its equivalents are added either directly to the fresh medium prior to starting cell culture or feeding it to the medium during cell culture, preferably during exponential phase growth in a growth medium. In case of feed addition, it should be taken into account that the effect of acetate takes place with some delay, i.e. a lag phase is observable with regard to the product protein yield enhancing effect. In general, addition of acetate via feed only is less effective. Addition of acetate directly to the cell culture medium, preferably a cell growth culture medium, prior to cell culturing and in the amounts stated above is strongly preferred according to the present invention, optionally in conjunction with further feed of acetate depending on the concentration. Most preferably, acetate is added only directly to the culture medium prior to culture onset, in particular at about 6-9 mM and in the form of sodium acetate, and is not replenished during cell cultivation via feed, preferably it is not replenished during culture growth in a suitable medium such as a high cell density growth medium.
'Addition to the medium prior to starting cell culture' according to the present invention means exposing cells at about the time of inoculation which includes the initial lag-phase before the onset of detectable growth or exposing them even prior to inoculation of the culture medium to acetate or its equivalents in the above stated amounts. Again, 'prior to inoculation' means that the inoculum pre-culture itself is grown in a medium comprising the acetate medium supplement in the above stated amounts. It is also possible to combine both aspects. In one particularly preferred embodiment, only the inoculum culture is treated with acetate in the amounts stated above whereas the cell culture growth medium

used for large-scale production culture is devoid of acetate salts in the >1mM range.

Suitable cells or cell lines can be any mammalian cell line. Suitable cell lines can be e.g. human cervical carcinoma (Hela) cells, chinese hamster ovary (CHO) cells such as e.g. the dhfr- CHO cell line DUK-BII (Chassin et al., PNAS 77, 1980, 4216-4220), SV-40 immortalized monkey kidney cells (COS-7) cells, canine kidney cells (MDCK), african green monkey kidney cells (VERO-76), baby hamster kidney (BHK) cells such as ATCC CCL10, human liver cells (Hep G2), lymphocytic cells (Jurkat T-cell line), hybridoma cells (e.g. SP2/0-Ag14, Shulman et al. 1977) or ‚myeloma' cells (such as e.g. NSO cells).

Suitable media and culture methods for mammalian cell lines are well-known in the art, as described in US 5633162 for instance. Examples of standard cell culture media for laboratory flask or low density cell culture and being adapted to the needs of particular cell types are for instance: Roswell Park Memorial Institute (RPMI) 1640 medium (Morre, G., The Journal of the American Medical Association, 199, p.519 f. 1967), L-15 medium (Leibovitz, A. et al., Amer. J. of Hygiene, 78, 1p.173 ff, 1963), Dulbecco's modified Eagle's medium (DMEM), Eagle's minimal essential medium (MEM), Ham's F12 medium (Ham, R. et al., Proc. Natl. Acad. Sc.53, p288 ff. 1965) or Iscoves' modified DMEM lacking albumin, transferrin and lecithin (Iscoves et al., J. Exp. med. 1, p. 923 ff., 1978). It is known that such culture media can be supplemented with fetal bovine serume (FBS, also called FCS), the latter providing a natural source of a plethora of hormones and growth factors. Cell culture of vertebrate and mammalian cells, respectively, has become a routine matter and is covered in detail e.g. in R. Ian Fresney, Culture of Animal cells, a manual, 4th e4dition, Wiley-Liss/N.Y., 2000.

Preferably, the cell culture medium according to the present invention is a medium allowing for and supporting growth of the animal cells thus cultured. Growth is understood as an increase in viable cell density during at least a certain period of cell culture. According to the present invention, such definition of 'growth medium' is to be understood as being opposed to the term 'maintenance medium' in its usual meaning in the art. A maintenance medium is a cell culture medium which supports cell viability but which does not encourage cell growth. Often, such maintenance media do not contain essential growth factors such as transferrin, insulin, albumin and the like.

Said embodiment of the medium being a mammalian cell culture medium comprising acetate or its equivalents in the afore mentioned amounts applies in particular to culturing or to a medium suited for the culture of lymphoid, preferably ‚myeloid', most preferably myeloma NSO cell line (such as e.g. cell line ECACC No. 85110503 and derivatives thereof, freely available from the European Collection of Cell Cultures (ECACC), Centre for Applied Microbiology & Research, Salisbury, Wiltshire SP4 0JG, United Kingdom). ‚Myeloid' cells are tumor cell lines of which NS0 is one example. NS0 cells are actually plasmacytomas, though being adressed in the art routinely and quite incorrectly as being ‚myeloid' cells (Barnes et al., Cytotechnology 32:109-123, 2000). Corresponding cell types are likewise particularly preferred embodiments in consequence. ‚Myeloma' NS0 cells have been found to potentially give rise to extremly high product yields, in particular if used for production of recombinant antibodies. Most standard NS0 cell lines are cholesterol-dependent, usually making cholesterol an obligate component of the culture medium. According to the present invention, lymphoid cells do comprise hybridoma cells generated by a number of techniques from antibody-secreting cells such as cell fusion with suitable tumor cell lines, including fusion with non-secreting hybridoma cell lines which gives rise to so-called triomas, or immortalisation with a transforming agent or virus as well as any other lymphoid cell line. In another preferred embodiment, however, a mammalian cell or cell line according to the present invention, is not a hybridoma cell line, meaning it is a non-hybridoma cell line, and more preferably it is a non-hybridoma, recombinant cell line, most preferably a recombinant ‚myeloma' cell line as defined above.

In a further prefered embodiment, the cell line is a NS0 cell line which is capable to express recombinant glutamine synthetase (GS). NS0 cells are specifically of advantage if used with the Glutamine synthetase (GS) expression system (Bebbington et al., 1992, High-level expression of a recombinant antibody from myeloma cells using a glutamine synthetase gene as an amplifiable selctable marker, Bio/Technology 10:169-175; Cockett et al., 1990, High level expression of tissue inhibitor of metalloproteinases in Chinese Hamster Ovary (CHO) cells using Glutamine synthetase gene amplification, Bio/Technology 8: 662-667). Preferably, the product protein gene sequence and the GS gene sequence are carried on a single GS plasmid vector for generating said transfected NS0 cell line, said genes either being expressed from different or same promoters

employing e.g. internal ribosome entry sites. - The GS-system is one of only two systems that are of particular importance for the production of therapeutic proteins. In comparison to the dihydrofolate reductase (DHFR) system, the GS system, and in particular the GS system used in combination with NS0 myeloma cells, offers a large time advantage during development because highly productive cell lines can often be created from the initial tranfection thus avoiding the need for multiple rounds of selection in the presence of increasing concentrations of selective agent in order to achieve gene amplification (Brown et al., 1992, Process development for the production of recombinant antibodies using the glutamine synthetase (GS) system, Cytotechnology 9:231-236). NS0 cells are phenotypically deficient in Glutamine-synthetase. Therefore the NS0 cell line which was derived from a mouse tumour cell line (Galfre, G. and Milstein,, C., Methods in Enzymol. 73, 3-75, 1981) is frequently the cell line of choice used in combination with the GS system at an industrial scale.
Further preferred, the cell line is a CHO cell line.

Preferably, the cell culture medium according to the present invention is devoid of fetal calf serum (FCS or FBS), which then is being termed 'serum-free'. Cells in serum-free medium generally require insulin and transferrin in a serum-free medium for optimal growth. Transferrin may at least partially be substituted by non-peptide chelating agents or siderophores such as tropolone as described in WO 94/02592 or increased levels of a source of anorganic iron favorably in conjunction with antioxidants such as vitamin C. Most cell lines require one or more of synthetic growth factors (comprising recombinant polypeptides), including e.g. epidermal growth factor (EGF), fibroblast growth factor (FGF), insulin like growth factors I and II (IGFI, IGFII), etc.. Other classes of factors which may be necessary include: prostaglandins, transport and binding proteins (e.g. ceruloplasmin, high and low density lipoproteins, bovine serum albumin (BSA)), hormones, including steroid-hormones, and fatty acids. Polypeptide factor testing is best done in a stepwise fashion testing new polypeptide factors in the presence of those found to be growth stimulatory. Those growth factors are synthetic or recombinant. There are several methodological approaches well-known in animal cell culture, an exemplary one being described in the following. The initial step is to obtain conditions where the cells will survive and/or will grow slowly for 3-6 days after transfer from serum-supplemented culture medium. In most cell types, this is at least in part a function of inoculum density.

Once the optimal hormone/growth factor/polypeptide supplement is found, the inoculum density required for survival will decrease.

In a more preferred embodiment, the cell culture medium according to the present invention is protein-free, more preferably it is a protein-free growth medium, that is it is free both of fetal serum and individual protein growth factors supplements or other protein such as recombinant transferrin or serum albumin for lipid binding and transport. Most preferably, it is a protein free medium as defined above but to which has been added recombinant or purified albumin or a sequence variant or fragment thereof. It should be noted, however, that even for NS0 cell lines which usually require cholesterol as a medium supplement, obtention of cholesterol-independent subspecies has been reported which can be continously cultured and grown for protein production (Lonza Biologics, UK). Protein-free culture media according to the present invention are particularly preferred in conjunction with the use of myeloma cell lines such as NS0.

A further preferred, possible embodiment of the method and the below specified cell culture medium of the present invention is high-density growth fermentation of the animal cells, e.g. in an industrial fed-batch bioreactor such as an airlift or perfusion bioreactor up to or beyond a viable cell density of up to or beyond 10^5, preferably 10^6, cells/ml. Consequently, a high-density growth culture medium has to be employed. Such high-density growth media can usually be supplemented with nutrients such as all amino acids, energy sources such as glucose in the range given above, inorganic salts, vitamins, trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), buffers, the four nucleosides or their corresponding nucleotides, antioxidants such as Glutathione (reduced), Vitamine C and other components such as important membrane lipids, e.g. cholesterol or phosphatidylcholine or lipid precursors, e.g. choline or inositol. A high-density medium will be enriched in most or all of these compounds, and will, except for the inorganic salts based on which the osmolarity of the essentially isotonic medium is regulated, comprise them in higher amounts (fortified) than the afore mentioned standard media as can be incurred from GB2251 249 in comparison with RPMI 1640. GB2251 249 gives examples of suitable high-density growth media. In accordance with the present invention, such high density cell culture medium comprises acetate in the amounts stated above, preferably in the absence of a butyrate. Further

preferred, a high-density culture medium according to the present invention is balancedly fortified in that a majority of amino acids except for Tryptophane are in excess of 75 mg/l in the culture medium. More preferably, in conjunction with the general amino acid requirement, the joint amounts of Glutamine and Asparagine are in total in excess of 1 g/l, most preferably in excess of 2 g/l of high-density culture medium. It goes without saying that the latter more preferred embodiment is less suitable in case of a recombinant cell line transfected with a Glutamine synthetase (GS) vector, in particular after rounds of amplification of the GS gene sequence have taken place. In those cells, an excess of e.g. glutamine jointly from exogenous and endogenous source would lead to production of ammonia, which is to be avoided.

In the context of the present invention, high-density cell culture is defined as a population of animal cells having temporarily a density of viable cells of at least or in excess of 10^5 cells/ml, preferably of at least or in excess of 10^6 cells/ml, and which population has been continously grown from a single cell or inoculum of lower viable cell density in a cell culture medium in a constant or increasing culture volume.

In a further prefered embodiment, the cell culture according to the present invention is a fed-batch culture wherein one or several other amino acids, preferably comprising at least Glutamin, is fed to the cell culture as described in GB2251 249 for maintaing their concentration in the medium and apart from controlling Glucose concentration by spearate feed. More preferably, the feed of Glutamin and optionally one or several other amino acids, ideally comprising Glutamine is combined with feeding one or more energy sources such as glucose to the cell culture as described in EP-229 809-A. Glutamine may at least be partly substituted by Asparagine (for substitution of glutamine by asparagine, see Kurano, N. et al., 1990, J. Biotechnology 15, 113-128) . Feed is usually initiated at 25-60 hours after start of the culture; for instance, it is useful to start feed when cells have reached a density of about 10^6 cells/ml. The amino acids that can be present in the feed are usually dosed in the range of from 10 to 300 mg total addition per amino acid per litre of culture volume; in particular glycine, lysine, arginine, valine, isoleucine and leucine are usually fed at higher amounts of at least 150 to 200 mg per L of culture volume as compared to the other amino acids. Except for GS cell lines, it can be of benefit to adjust the total feed of glutamine and/or asparagine to the range of from 0.5 to 3 g per L of culture volume, preferably to the range of from 1 to 2 g per L of culture volume. The feed

can be added as shot-addition or as contionusly pumped feed, preferably the feed is almost continously pumped into the bioreactor. It goes without saying that the pH is carefully controlled during fed-batch cultivation in a bioreactor at an approximately physiological pH optimal for a given cell line by addition of base or buffer. When glucose is used as an energy source the feed of glucose feed is adjusted usually to keep glucose concentration of the medium of from 1 to 10 grams, preferably of from 3 to 6 grams per litre of the culture. Apart from inclusion of amino acids, the feed preferably comprises a low amount of choline in the range of 5 to 20 mg per litre of culture.

A corresponding cell culture medium comprising an acetate supplement and a medium concentrate for preparing such medium are futher objects of the present invention. The above description applies likewise to these embodiments of the present invention.

Experiments

The GS-NS0 cell line 6A1(100)3 secreting recombinant mouse/human chimeric IgG cB72.3 (Bebbington, 1992) was used in all experiments. Cell culture was either carried out in shake flask culture or in a fed-batch mode in a 10 l standard airlift bioreactor, essentially as described (Bebbington 1992). Feeds essentially comprised amino acids and carbohydrate as described above for high-density media and fermentation, respectively. Feed volume was at 4% of post-inocculation volume. Feed was initated at a rate of 0.2 ml /Lh when viable cell density exceeded $14x10^5$ cells/ml. Temperature was controlled at 36.5 degree centigrade; for airlift, air saturation was at 15%. pH was controlled at pH 7.00. Culture was inocculated at $2x10^5$ viable cells/ml. Medium is protein-free growth medium ProCHO4-CDM (Biowhittaker, UK) supplemented with 50 mM methionine sulfoximine (MSX). Increases in protein yield of up to 98% (from 470 mg/L of the control as compared to 768 mg/L for a strain grown with 10 mM acetate) were observed. Acetate was found not to suppress growth. It did slighrly decrease growth rate but did not lower maximum viable cell densities. Positively, it did prolong stationary phase upon fermentation.

Cumulative cell time (10^6 cell /h ml) was calculated by integration of the cell growth curve, essentially as described by Renard et al. (1988, Biotechnology Letters 10, 9 1-96).

Fig. 1 shows the effect of potassium and sodium acetate on productivity in fed-batch shake flask culture. Either sodium acetate or potassium acetate were added to shake flask culture during mid-exponential growth phase to the final concentrations stated in the respective sections of the table of Fig. 1. Cultures were counted daily by Trypan blue method and samples were taken for product analysis by means of protein A fractionation and HPLC analysis. Sodium acetate at 10 mM performed even better than potassium acetate at either 10 mM or 15 mM. The CCT was similar in all treatments (including control). Consequently, single cell productivity (q_p) was found to be increased.

Fig. 2 shows growth profiles for fed-batch bioreactor fermentations containing no or 10 mM sodium actetate. Duplicate cultures both for control and 10 mM sodium acetate supplemented culture were tested for viable cell concentration (determined by Trypan blue exclusion standard counting test). Feed was started at day 4. The acetate was not fed during growth phase as in Fig. 1 but was included in cell culture production medium right away to which the inoculum cells were added. As can be seen, the yield enhancing effect of acetate proved to be highly reproducible (Fig. 3) as was growth behavior. No significant growth suppression could be observed in terms of maximum cell density achievable. Growth rate was not substantially decreased though a certain effect of acetate addition was reproducibly found. The CCT remained essentially similiar for all cultures.

In an additional set of experiments, the feed was further supplemented with LiCl both for the control and the culture treated with acetate as said in the preceding paragraph, accumulating to a final concentration of approx. 1mM (data not shown). The addition of a Li-salt did not prove to modify the effect of acetate addition.

Fig. 4 compares the effect of inclusion of sodium acetate in inoculum cultures (in inoc) or at the time of inoculation to the production medium (at inoc) or in the feed given during mid-exponential phase (at feed) or combinations thereof in fed-batch shake flask culture. All values are expressed as percent difference compared to control. A clear dose dependency was observable as regards the amount of acetate added. Addition of acetate to the inoculum culture or at the time of inoculation, meaning the use of an acetate supplemented culture medium for production culture, was found to be the most effective mode in view of increased yield and maximized single cell productivity.

Claims

1. Method of producing a product protein, wherein the protein is expressed from mammalian cell in cell culture at least during a certain span of time during cell culture, comprising the steps
   e) preparing a cell culture medium for culturing mammalian cells, preferably preparing a cell culture medium that is devoid of butyrate, and further preferred preparing a cell culture medium allowing for growth of the mammalian cells, more preferably a protein-free cell culture growth medium,
   f) and further adding acetic acid or an acetate salt or an acetyl ester to a final concentration of from 1 to 20 mM, preferably of from 3 to 15 mM, more preferably of from 5 to 12 mM, most preferably of from 6 to 9.5 mM, said addition being carried out either directly to the medium prior to starting cell culture or feeding it to the medium during cell culture,
   g) further culturing, preferably growing, the cell in said medium with concomittant expression of product protein,
   h) and finally harvesting said protein from the cell culture.

2. Method according to claim 1, characterised in the the addition of acetic acid or a salt therof is carried out directly to the medium prior to or at starting the cell culture.

3. Method according to one of the preceding claims, characterised in that an acetate alkali metal or alkaline earth metal salt is added to the medium with the proviso that the the alcaline metal is not lithium.

4. Method according to one of the preceding claims, characterised in that the cells are NS0 cells.

5. Method according to claim 4, characterised in the cells are NS0 cells that are recombinant for and can express Glutamine synthetase (GS).

6. Cell culture medium for animal cell culture, characterised in that the medium is suited

for culturing mammalian cells and comprises acetic acid or an acetate salt or a biologically activated acetyl ester at a concentration of from 1 to 20 mM, preferably of from 3 to 15 mM, more preferably of from 5 to 12 mM, most preferably at about 6 to 9.5 mM, and preferably is devoid of butyric acid or any of its salts.

7. Cell culture medium according to claim 3, characterised in that the medium is a high density cell culture medium.

8. Cell culture medium according to claim 6 or 7, characterised in that the medium is a cell culture medium allowing for growth of animal cells.

9. Cell culture medium according to claims 6-8, characterised in that the medium is a serum-free and protein-free cell culture medium, preferably a protein-free medium suitable for NS0 cell culture.

10. A medium concentrate for preparation of a culture medium as defined in claim 6 which is either a solid or a liquid.

Abstract

A method for culturing cells in the presence of an alcanoic acid for enhancing protein production.

RGI/March 2002

Parameter	Control	Potassium acetate (mM)			Sodium acetate (10 mM)
		5	10	15	
CCT (10^6 cell h mL^{-1})	554	597 (8%)	593 (7%)	572* (3%)	535 (-3%)
Product (mg L^{-1})	365	448 (23%)	480 (32%)	487* (33%)	501 (37%)
q_P (mg 10^6 $cells^{-1}$ h^{-1})	0.659	0.752 (14%)	0.810 (23%)	0.852 (29%)	0.937 (42%)

- *A duplicate culture exhibited very poor cell growth. No product concentration was determined.

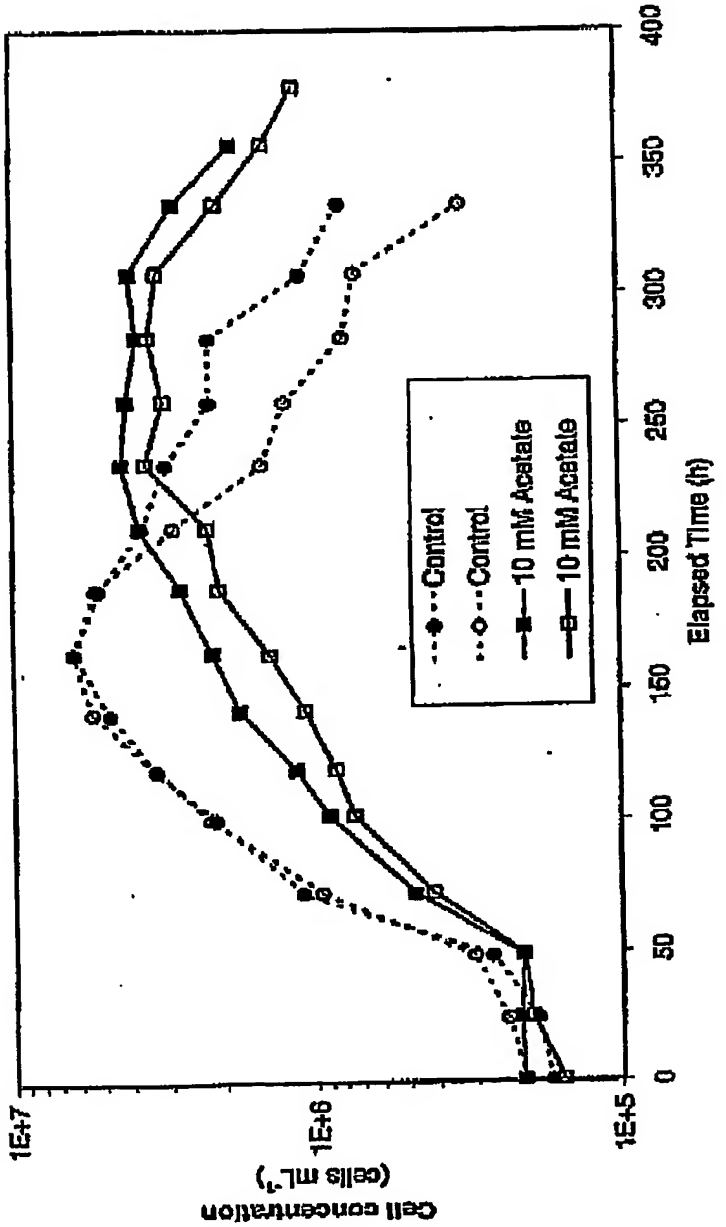
Cell concentration
(cells mL-1)
1E+7
1E+6
1E+5
0
50
100
150
200
250
300
350
400
Elapsed Time (h)
Control
Control
10 mM Acetate
10 mM Acetate

Fig. 2

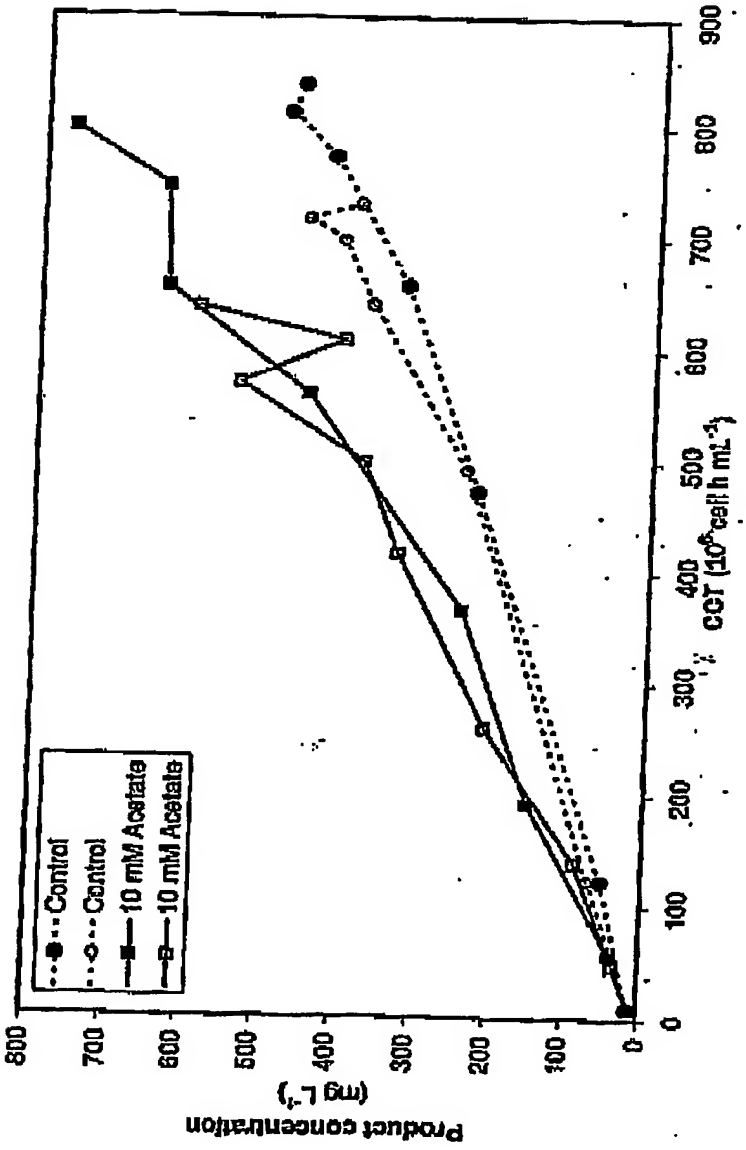
Product concentration
(mg L-1)
CCT (10^6 cell h mL-1)
Control
Control
10 mM Acetate
10 mM Acetate
0
100
200
300
400
500
600
700
800
900

Fig. 1

Fig. 4

Treatment	Maximum viable cell concentration	CCT	Product concentration	q_P
No acetate (Control)	0%	0%	0%	0%
5mM in inoculum	-4%	23%	24%	12%
10mM in inoculum	24%	40%	97%	56%
5mM at feed	8%	-13%	-15%	-5%
10mM at feed	1%	-8%	8%	17%
15mM at feed	26%	7%	1%	-3%
5mM in inoc - 10mM at inoc	9%	59%	98%	39%
5mM in inoc - 10mM at feed	12%	47%	73%	28%